ABSTRACT

Fresh commercial goats' milks were examined for their lipid contents and distribution of these lipids among milk fractions. Whole milk, skim milk (produced by centrifugation at 330 and 2,000 × *g*), and cream were studied. Petroleum ether (free lipids) and chloroform methanol (2:1) (bound lipids) were used successively to extract the lipids from all milk fractions. Average total lipid content for five bulk milk samples was 5.0 ± 1.2%. Lipid fractions of whole milk and cream contained 97 to 99% free lipid and 1 to 3% bound lipid, respectively. Free lipid was 96.8% triglyceride, whereas bound lipids contained neutral lipid, glycolipid, and phospholipid. In this respect, goats' milk resembled cows' milk. However, goats' skim milk fractions contained significantly more free lipid than did cows' milk. This free lipid, investigated in detail by gas chromatography, was shown similar in triglyceride distribution and fatty acid content to whole goats' milk triglyceride. Quantitative data for the triglyceride distribution in all fractions are given and differ from published data for fresh goats' milk.

INTRODUCTION

It often has been stated that goats' milk is "naturally homogenized" and that goat cream does not rise. This claim is based in part on studies (4, 8) on warm milks that were chilled rapidly. Observations in our laboratory showed that if commercial bulk goats' milk was stored overnight in a refrigerator, a noticeable layer of cream formed. This observation prompted us to investigate how goats' milk behaves upon centrifugation, in comparison with cows milk, and to determine the lipid distribution between the cream and skim milk layers formed by centrifugation. The amount of free and bound lipids in each fraction and in whole milk was determined. Various types of lipids were examined qualitatively by thin layer chromatography and quantitatively by silicic acid column chromatography. Neutral fat also was analyzed by gas liquid chromatography.

Received September 28, 1981.

[1] Agricultural Research Service, US Department of Agriculture.

EXPERIMENTAL PROCEDURE

Raw goats' milk samples were obtained from a commercial dairy-goat company. The milk was maintained at 5°C until used. For comparison, a cows' milk sample (pooled from 60 cows, several breeds) was obtained from a local farm.

Centrifugation

Whole milk was separated in a laboratory centrifuge (International Equipment Company) for 20 min at 20°C at 1200 rpm (330 × *g*) and 3000 rpm (2000 × *g*). The centrifuged samples were cooled to solidify the cream layer, which was removed carefully. The skim milk and cream fractions then were freeze-dried and extracted (1).

Lipid Extraction

Free lipids were obtained by extracting the freeze-dried samples four times with Nanograde petroleum ether (30 to 65 bp); the bound lipids were obtained by three subsequent extractions with chloroform-methanol (2:1, vol/vol) as in (1). The scheme is given in Figure 1.

Lipid extracts were analyzed by thin-layer chromatography (TLC) (1). The developing solvents were petroleum ether-diethylether-acetic acid (90:10:1, vol/vol/vol) for neutral lipids and chloroform-methanol-water (65:25:4, vol/vol/vol) for polar lipids (glycolipids and phospholipids). Iodine vapor was used to visualize the lipids.

Silicic Acid Column Separations. Silicic acid (100 to 200 mesh) that was activated by

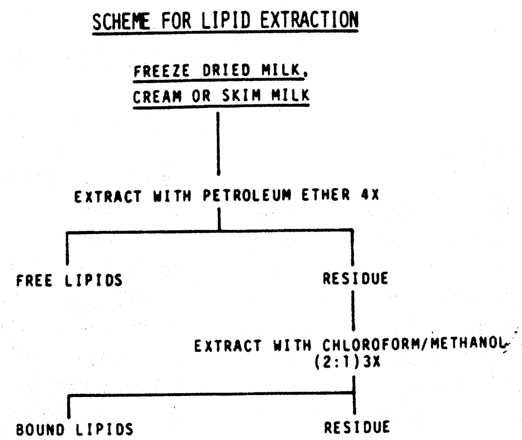

Figure 1. Scheme for lipid extraction from freeze dried products. The terms bound and free refer to those lipids that are extractable by this process.

treatment at 110°C for 12 h was used for all column separations. Free lipids were separated as in (1). Bound lipids were separated by the modified procedure of Masoro et al. (7): Fraction A, eluted with chloroform, contains neutral lipids; Fraction B, eluted with acetone, contains glycolipids; Fraction C, eluted with chloroform-methanol (6:1, vol/vol), contains phosphatidylethanolamine; Fraction D, eluted with ethylacetate-methanol (3.5:2, vol/vol), contains phosphatidylserine and phosphatidylinositol; Fraction E, eluted with chloroform-methanol 1:1, vol/vol), contains most of phosphatidylcholine; Fraction F, eluted with methanol, contains phosphatidylcholine and sphingomyelin. Each fraction was purified again by the same solvent systems. Each separation step was analyzed by TLC.

Gas-Liquid Chromatography

Gas-liquid chromatography of intact triglycerides of the free and bound fractions was on the Perkin-Elmer Model 900 Gas Chromatograph (Perkin-Elmer Corp., Norwalk, CT) equipped with a flame ionization detector. The triglycerides were separated on a 50 × .32 cm siliconized stainless steel column packed with 2% SE 30 on Gas Chrom G. The flash heater and manifold were maintained at 280 and 350°C, respectively. The column temperature was programmed from 150 to 330°C at 6.5°C per min. Helium was the carrier gas.

Transmethylation of the triglyceride fractions was accomplished with potassium methylate by the procedure of Schwartz (13). Approximately 200 μg of the triglyceride in 100 μl of hexane was added to 100 mg of the celite-potassium methylate mixture packed in a .5 × 4 cm chromatographic column (prepared from a disposable pasteur pipette). The methyl esters were eluted with 500 μl of CS_2, and an aliquot was injected directly into the Perkin Elmer Gas Chromatograph. The C_6 through C_{18} methyl esters were separated on a 1.83 m × .32 cm stainless steel column packed with 7.5% stabilized ethylene glycol adipate plus 2% phosphoric acid. The flash heater and manifold were maintained at 200°C, and helium served as the carrier gas. The column temperature was programmed from 80 to 140°C at 4°C/min and held at 140°C to accomplish separation of the C_{18} methyl esters.

Quantitation of the triglyceride and fatty acid compositions of the free and bound fractions was accomplished by triangulation of the GLC peaks.

RESULTS AND DISCUSSION

Lipid Content of Goats' Milk

The total lipid content of the goats' milk samples was variable (3.7 to 6.7%) as in Table 1. Milk samples were obtained during the spring of the year from a commercial goat's milk processor but the numbers of breeds and herds involved are not known. Similar variation of milk lipid content also was observed among breeds of dairy cattle (2); however, the variation of lipid content was much greater in these goats' milks than in cows' milk (2, 5).

Centrifugation of goats' and cows' milks yielded cream and skim milk fractions; their lipid contents are compared in Table 1. Centrifugation of goats' milk at the lower speed (1200 rpm) left more of the total lipids (6.5%) in the skim milk fraction as compared to cows milk (3.0%). The reported smaller size of the fat globules in goat milk may be related to this difference (4, 8, 12). Centrifugation of the milks at higher speeds (3000 rpm) gave almost identical results for goats' and cows' milks (1.7 and 1.2%, respectively, of total lipid left in the skim milk).

TABLE 1. Distribution of lipids between cream and skim milk fractions of goats' milk.

Sample	% Total lipids in milk	% Total lipids in Cream 1200 rpm	Cream 3000 rpm	Skim milk 1200 rpm	Skim milk 3000 rpm
3[a]	3.7	94.2	97.4	5.8	2.6
4	4.7	93.0	99.3	7.0	.7
5	4.2	97.2	98.2	2.8	1.8
6	6.7	91.3	99.2	8.7	.8
7	5.7	91.7	97.6	8.3	2.4
Average ± SD	5.0 ± 1.2	93.5 ± 2.4	98.3 ± .9	6.15 ± 2.3	1.7 ± .9
Cows bulk[b] milk (60 cows)	4.1	97.0	98.8	3.0	1.2

[a]Samples 3 through 7 were pooled milks, which had been bulked and cooled to 5°C, and were from a commercial dairy goat processor.

[b]Fresh bulk milk obtained after cooling to 5°C.

Distribution of Lipids in Goats' Milk

The distribution of free (petroleum ether extractable) and bound (chloroform-methanol (2:1) extractable) lipids in the goats' milk samples was examined. In whole goats' milk the bound lipids (Table 2) averaged 3.2% as compared to 2.7% for the pooled cows' milk. Although the overall lipid content of the goats' milk samples (Table 1) varied considerably, the ratio of bound to free lipids remained constant. In a similar fashion, the cream fraction of the goats' milk had a relatively constant ratio of bound to free lipid which is also in accord with data from cows' milk. The goats' skim milk fractions, however, contained almost twice the free lipid content of the cows' skim milk. This observation will be discussed in detail below.

TABLE 2. Free and bound lipids in milk fractions reported as percent of total lipid.

Sample		Whole milk	Cream 1200 rpm	Cream 3000 rpm	Skim milk 1200 rpm	Skim milk 3000 rpm
3	Free	97.3	98.0	99.4	73.0	85.7
	Bound	2.7	2.0	.6	27.0	14.3
4	Free	95.7	97.8	99.2	62.5	90.7
	Bound	4.3	2.2	.8	37.5	9.3
5	Free	97.1	98.0	98.7	71.5	66.7
	Bound	2.9	2.0	1.3	28.5	33.3
6	Free	97.5	98.2	98.9	82.4	75.0
	Bound	2.5	1.8	1.1	17.6	25.0
7	Free	96.4	98.3	98.3	89.7	85.7
	Bound	3.6	1.7	1.7	10.3	14.5
Average	Free	96.8 ± .7	98.1 ± .2	98.9 ± .4	75.8 ± 10.5	80.7 ± 9.7
± SD	Bound	3.2 ± .7	1.9 ± .2	1.1 ± .4	24.2 ± 10.5	19.3 ± 9.7
Cows' bulk	Free	97.3	98.8	98.4	43.2	41.1
milk	Bound	2.7	1.2	1.6	56.8	58.9

All lipid extracts (Table 2) were examined by TLC. The free lipid (petroleum ether-extractable) fractions did not contain any polar lipids, as with cows' milk (1). All of the neutral lipid fractions contained tri-, di-, and mono-glycerides and cholesterol as cows' milks (1). The bound lipid (chloroform-methanol 2:1-extractable) fractions contained qualitatively all of the neutral lipid components as well as the polar lipids. Polar lipid compositions for all of the milk samples were not identical.

All goats' milk samples contained cerebroside monohexoside, cerebroside dihexoside, phosphatidylethanolamine, phosphatidylcholine, phosphatidylserine, phosphatidylinositol, and sphingomyelin, as cows' milk (3). Some milk samples contained lysocompounds of phosphatidylethanolamine, phosphatidylcholine, phosphatidylserine, and phosphatidylinositiol. One sample of goat milk contained over 14 polar lipid components; some were not identified. What caused this variation is not known.

Quantitative Composition of Goats' Milk Lipids

All goats' whole milk samples (Table 2) were combined in equal volumes and their free and bound lipid contents quantitated by silicic acid columns, as in (1, 7). The composition of free lipids (Table 3) was: triglycerides, 96.9%, diglycerides, 2.2%, and monoglycerides, .9%. A similar composition was observed also in cows' milk (1). The combined bound lipid fraction contained neutral fat, 46.8%, glycolipid, 8.5%, and phospholipids, 44.7%. The composition of bound neutral fat fraction was: triglycerides, 56.7%, diglycerides, cholesterol and free fatty acids, 33.3%, and monoglycerides, 10.0%. The phospholipid distribution was determined on a milk sample which contained no lysophospholipid components (see above). The results are in Table 4. This distribution is similar to that published by Patton et al. (11) for the phospholipid distribution of an individual animal's milk.

TABLE 3. Quantitative distribution of lipids in bound and free fractions of goats milk.

Free lipids[a]	
96.8%	Triglycerides
2.2%	Diglycerides
.9%	Monoglycerides
Bound lipids	
46.8%	Neutral lipid
8.5%	Glycolipid
44.7%	Phospholipid

[a]These fractions were obtained by pooling equal volumes of whole milks from samples 3 through 7 (Table 2).

Distribution and Nature of Triglycerides in Goats' Milk

The most striking difference between goats' and cows' milks was in the skim milk fractions. Even in the 3000 rpm skim milks (Table 2), where cows' and goats' milks gave nearly equivalent total lipid contents, the free lipids predominate in goats' milk. These fractions also exhibited the most variation from sample to sample. Patton et al. (10) showed that on aging goat milk for 1 day at 2 to 4°C, there were substantial increases in phospholipid and cholesterol contents of the skim milk phase, indicating that cooled goats' milk fat globules

TABLE 4. Quantitative analysis of phospholipids of the bound lipid fraction of goats' milk.

	% Phospholipid	
	This study	Patton et al. (11)
Phosphatidyl ethanolamine	35.4	25.5
Phosphatidyl serine	3.2	9.6
Phosphatidyl inositol	4.0	1.4
Phosphatidyl choline	28.2	27.6
Sphingomyelin	29.2	35.9

may be more susceptible to damage. Correspondingly more neutral lipid also might be expected in the skim milk as well. Thus, excess free lipids in the goat skim milk relative to cows' milk could account for some of the problems in variability of milk quality.

Skim milk was produced by centrifugation of pooled goats' milk at 1200 rpm; 95.1% of the total lipid content was in the cream and 4.9% remained in the skim milk. The skim milk fraction was separated into casein and whey by adjusting the pH to 4.2 with HCl. Both fractions were examined for lipids. Distribution of total lipids between the casein and whey fractions was 71 and 29%. Bound lipid distribution was 89.4% in casein and 10.6% in whey fractions, whereas the free lipid distribution was 53.8% in casein and 46.2% in whey fractions. Thus, the free lipids, which were higher (Table 2) in goats' skim milk than in cows' skim milk, are distributed evenly between casein and whey fractions.

The nature of the lipids associated with the goat skim milk fraction was investigated. Because triglycerides (Table 3) represent the bulk of the free lipid fraction, an experiment was conducted on pooled goats' milk to determine the distribution and composition of triglycerides associated with bound and free lipids of skim and cream. Triglycerides were isolated from each fraction by silicic acid chromatography and analyzed for their average carbon numbers (Table 5). Analysis of variance showed there are no statistically significant differences in the overall triglycerides for carbon numbers 28, 32, 38, 40, 42, 44, 52, and 54. These triglycerides represent >60% by weight of each of their respective fractions. Duncan's multiple range test showed that four of the seven cases where the means were different could be accounted for by variance in either the free or bound triglycerides of the cream fraction (Table 5). The skim milk triglycerides, whether free or bound, do not appear to vary from the composite. When the data were grouped as to source (cream or skim) only triglycerides with a carbon number of 26 were significantly different ($P = .008$) between the skim milk and cream fractions. Thus, the cream fraction had a higher content of this class of triglyceride; all others were not significantly different. When the data were grouped by extraction solvent (bound or free), only triglycerides with a carbon number of 30 were significantly different. The remaining

TABLE 5. Analysis of carbon number of triglycerides associated with the free and bound lipids of cream and skim milk fractions of goats' milk.

	Weight %[1]					
Carbon[b] number	Cream free	Cream bound	Skim free	Skim bound	Composite $\bar{X}$	Composite SD
26	.49^x	.60^x	.39	.41	.47	.09
28	1.10^x	1.32	.93^x	1.10	1.11	.21
30	2.00	2.55	2.11	2.24	2.22	.20
32	3.70	4.00	3.83	4.12	3.91	.33
34	5.32^x	6.12	6.00	6.10	5.88	.24
36	9.12	9.82^x	9.35	9.35	9.41	.27
38	12.4	12.7	12.7	12.4	12.6	.4
40	14.0	13.9	14.0	13.9	13.9	.6
42	11.7	12.0	12.1	11.9	11.9	.4
44	9.98	10.2	10.3	10.1	10.1	.3
46	7.55^x	6.73^y	7.14	7.10	7.13	.12
48	5.21	4.66^x	5.00	5.29	5.04	.30
50	6.65^x	5.84	6.08	6.06	6.16	.28
52	6.68	6.18	6.40	6.38	6.45	.36
54	3.61	3.51	3.61	3.67	3.70	.31

^{x,y}For each row means which have a letter superscript are significantly different; two means with the same superscript are not different from each other. Means with no letters are not significantly different.

[1] Each is the mean of four determinations.

TABLE 6. Comparison of carbon number for triglycerides of goats' milk.

Carbon number	Weight %		
	Bulk milk this study composite	Individual[a] animal milk	Canned[b] goats' milk
26	.47	.06	.46
28	1.11	.18	.99
30	2.22	.48	2.04
32	3.91	1.1	3.32
34	5.88	3.0	5.25
36	9.41	7.0	8.75
38	12.6	10.8	11.9
40	13.9	9.6	12.1
42	11.9	7.8	10.2
44	10.1	7.5	8.96
46	7.13	6.2	6.97
48	5.04	8.4	5.62
50	6.16	14.1	7.80
52	6.45	16.0	9.38
54	3.50	7.7	6.31

[a]Calculated to weight percent from data of Marai et al. (6).

[b]Taken from data of Parodi (9).

TABLE 7. Analysis of fatty acid distribution of triglycerides associated with the free and bound lipids of cream and skim milk fractions of goats' milk.

Me ester	Weight %				Average	
	Cream free	Cream bound	Skim free	Skim bound	$\bar{X}$	SD
C_6	2.39	2.08	2.85	2.49	2.45	.31
C_8	3.49	3.13	3.51	3.64	3.44	.21
C_{10}	12.6	10.5	11.6	10.4	11.3	1.0
C_{12}	4.41	5.31	5.12	5.16	5.00	.40
C_{14}	11.5	11.9	12.4	12.5	12.1	.48
C_{16}	27.4	28.4	27.2	27.7	27.8	.53
$C_{18:0}$	8.36	8.23	6.83	7.56	7.74	.70
$C_{18:1}$	26.8	28.2	27.7	27.6	27.6	.6
$C_{18:2}$	3.03	2.19	2.75	2.84	2.70	.36

class (carbon number = 48) showed no pattern. Thus, with the exceptions noted above, the majority of classes of triglycerides showed no significant differences.

Averages for the triglyceride profile were compared to those reported (Table 6). The averages showed a substantially higher percentage of lower carbon number triglycerides than Marai et al. (6) for fresh cream from a single animal. However, our study on fresh bulk milk is in relative agreement with carbon numbers for canned goats' milk by Parodi (9) and, thus, may be more representative of pooled milks.

Fatty acid profiles of triglycerides of the bound and free fractions of cream and skim milk also were determined (Table 7), and no differences were significant. Averages for fatty acid profiles agree with averages of (8). Thus, the excess free lipid in goat skim milk represents primarily triglycerides, which have little or no different distribution of either carbon number or fatty acid content. There does not appear to be a preponderance of shorter chain acids in these skim milk lipids (either free or bound) as compared to the cream fraction. However, the free lipid fraction does represent a significant difference between goats' and cows' skim milks.

REFERENCES

1 Cerbulis, J. 1967. Distribution of lipids in various fractions of cow's milk. J. Agric. Food Chem. 15:784.

2 Cerbulis, J., and H. M. Farrell, Jr. 1975. Composition of milks of dairy cattle. I. Protein, lactose, and fat contents and distribution of protein fraction. J. Dairy Sci. 58:817.

3 Easter, D. J., S. Patton, and R. D. McCarthy. 1971. Metabolism of phospholipid in mammary gland: I. The supply of phospholipid for milk synthesis in the rat and goat. Lipids 6:844.

4 Jenness, R., and S. Parkash. 1971. Lack of a fat globule clustering agent in goats' milk. J. Dairy Sci. 54:123.

5 Lythgoe, H. C. 1940. Composition of goat milk of known purity. J. Dairy Sci. 23:1097.

6 Marai, L., W. C. Breckenridge, and A. Kuksis. 1969. Specific distribution of fatty acids in the milk fat triglycerides of goat and sheep. Lipids 4:562.

7 Masoro, E. J., L. B. Rowell, and R. M. McDonald. 1964. Skeletal muscle lipids. I. Analytical method and composition of monkey gastrocremius and soleus muscles. Biochim. Biophys. Acta 84:493.

8 Parkash, S., and R. Jenness. 1968. The composition and characteristics of goat's milk: A review. Dairy Sci. Abstr. 30:67.

9 Parodi, P. W. 1973. Detection of synthetic and adulterated butterfat. 4. GLC triglyceride analysis. Australian J. Dairy Technol. 28:38.

10 Patton, S., C. Long, and T. Sokka. 1980. Effect of storing milk on cholesterol and phospholipid of skim milk. J. Dairy Sci. 63:697.

11 Patton, S., B. H. Stemberger, and C. M. Knudson. 1977. The suppression of milk fat globule secretion by colchicine: an effect coupled to inhibition of exocytosis. Biochim. Biophys. Acta 499:404.

12 Schultz, E. W., and L. R. Chandler. 1921. The size of fat globules in goat's milk. J. Biol. Chem. 46:133.

13 Schwartz, D. P. 1977. Methods for the isolation and characterization of constituents of natural products. XXI. Use of a Celite-potassium methylate column for rapid preparation of methyl esters from microgram amounts of glycerides. Microchem. J. 22:457.